Case report
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The First Korean case of combined oxidative phosphorylation deficiency-17 diagnosed by clinical and molecular investigation

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Combined oxidative phosphorylation deficiency-17 (COXPD-17) is very rare and is caused by homozygous or compound heterozygous mutations in the *ELAC2* gene on chromosome 17p12. The *ELAC2* gene functions as a mitochondrial tRNA processing gene, and only 4 different pathogenic mutations have been reported in *ELAC2*-associated mitochondrial dysfunction involving oxidative phosphorylation. Affected patients show various clinical symptoms and prognosis, depending on the genotype. We report a novel mutation in the *ELAC2* gene (c.95C>G [p.Pro32Arg], *het*), in an infant with COXPD-17 who presented with encephalopathy including central apnea and intractable epilepsy, and growth and developmental retardation. During hospitalization, consistently elevated serum lactic acid levels were noted, indicative of mitochondrial dysfunction. The patient suddenly died of shock of unknown cause at 5 months of age. This is the first case report of COXPD-17 in Korea and was diagnosed based on clinical characteristics and genetic analysis.

Key words: *ELAC2*, Oxidative phosphorylation, Encephalopathy, Hyperlactatemia

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Introduction

Mitochondrial diseases are heterogeneous disorders, causing varying degrees of impairment in the production of adenosine triphosphate through oxidative phosphorylation (OXPHOS) by the mitochondrial respiratory chain complexes[1]. Mitochondria are under dual genetic control, from mitochondrial DNA (mtDNA) itself and nuclear DNA, and respiratory chain enzyme complexes of the OXPHOS system are composed of more than 70 subunits encoded by nuclear genome and 13 subunits encoded by mtDNA[2,3]. Isolated or combined enzyme complex deficiency presents a wide variety of clinical manifestations, and involves various inheritance patterns such as Mendelian or mitochondrial genetics, which could interfere with establishing a clear genotype of mitochondrial diseases[1].

Combined oxidative phosphorylation deficiency-17 (COXPD-17) is caused by homozygous or compound heterozygous mutations in the *ELAC2* gene on chromosome 17p12 (MIM No.615440). Although several mutations of *ELAC2* was reported in relation to prostate cancer[4], to date, only 1 compound heterozygous mutation and 3 homozygous mutations in the *ELAC2* gene associated with impaired OXPHOS have been found worldwide[5-7]: c.631C>T;1559C>T(p.Arg211*;Thr520Ile), c.460T>C(p.Phe154Leu), c.1267C>T (p.Leu423 Phe), c.1423+2T>A.

Here, we present a patient with COXPD-17 associated with a novel mutation in the *ELAC2* gene who exhibited central apnea, intractable epilepsy, and growth and developmental retardation.

Case report

This female patient was born at 38 weeks of gestation by caesarean section due to a fetal heart defect detected on antenatal sonography. Her mother was healthy during pregnancy without significant medical history. There was no remarkable history in each parent's family and the patient was their first baby. She showed intrauterine growth retardation. Her weight (2,350 g), height (46 cm), and head circumference (31.5 cm) were below 10th percentile. She had subtle dysmorphic features including hypertelorism, high arched palate, and both simian creases.

From the second day of birth, she presented apnea more than four times a day and her percutaneous oxygen saturation decreased during feeding. To maintain airway and support respiration, she was intubated and received mechanical ventilator therapy. For cardiac anomaly of tetralogy of Fallot, total correction operation was performed on the 36th day of hospitalization, earlier than that planned for 3 months of age, because the apnea had worsened. Despite the fact that the cardiac anomaly was corrected, sudden frequent desaturation was aggravated, and frequent seizure like motions were noticed. She had 2 types of clinical seizures several times a day, as follows: (1) sudden attack of desaturation with tonic seizures of both extremities lasting for 30 seconds to 1 minute, and (2) upper eyeball deviation with smacking her lips followed by tonic seizure of both extremities with desaturation lasting for 2 to 3 minutes. Her seizures were medically intractable, despite the adequate dose of several antiepileptic drugs including phenobarbital, topiramate, levetiracetam, vigabatrin, valproate, oxcarbazepine, zonisamide, and prednisolone.

At the age of 3 months, growth parameters such as height (53 cm), weight (4.4 kg), and head circumference (38 cm) were all below the 5th percentile. A global developmental delay seemed obvious, because she rarely showed spontaneous eye opening and movement of limbs, and did not show appropriate responses such as avoidance or crying to external stimuli. She also had muscle hypotonia. Central apnea was strongly suspected because there was no effort of spontaneous breathing by the patient. Repeated electroencephalography studies demonstrated poorly organized sleep feature, and nearly continuous asynchronous diffuse slow waves mixed with very frequent multifocal independent spike foci over the bilateral frontal and bilateral posterior head regions. The brain magnetic resonance imaging showed no clinically relevant finding.

After reviewing the medical records again, it was recognized that her arterial lactic acid levels were consistently elevated above normal levels (2.3–5.0 mmol/L, normal range<1.6 mmol/L) (Fig. 1). We further investigated, with the focus on hyperlactatemia. The results of investigations for inborn error of metabolism including tandem mass spectrometry, serum amino acid, and urine organic acid analysis, revealed no significant abnormalities. However, a blood lactic acid level of 3.8 mmol/L and pyruvic acid level of 1.3 mg/dL was found when she was in a stable condition, without any hypoxic sign. The cerebrospinal fluid lactic acid level was also elevated to 7.6 mmol/L. All characteristics and measurements were indicative of mitochondrial disease. She was supplemented with coenzyme Q10 (5 mg/kg/day), thiamine (30 mg/day), and L-carnitine (100 mg/kg/day). Although she suffered from intractable epilepsy, she was transferred to the ward in a stable condition, with a home mechanical ventilator and a feeding tube inserted through gastrostomy, at 5 months of age. However, a few days later, she suddenly showed hypotension with severe metabolic and lactic acidosis, and despite aggressive resuscitation, she died after 18 hours. As a cause of death, cardiomyopathy was excluded as a normal result of echocardiogram performed several days before her death. But, sepsis was suspected because she had an increased total leukocyte count of 41,110 cells/μL (segmented neutrophil 50%) and elevated C-reactive protein level of 13.9 mg/dL, on the day of the event, even though there were no causative microorganism were identified.

For genetic analysis, a written consent was obtained from the family. Her karyotype was normal (46, XX) and the array comparative genomic hybridization also showed no abnormalities. For

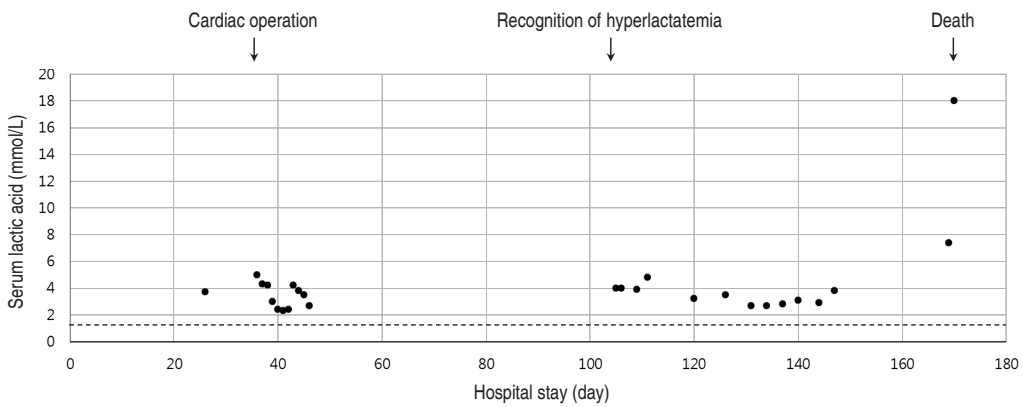

Fig. 1. Serum lactic acid levels during hospitalization. Serum lactic acid levels were consistently above the upper normal limit (---) and varied with the patient's condition.

the next step, targeted exome sequencing (TES) was carried out in using the NextSeq 500 sequencing platform and TruSight One Sequencing panel (Depth 94X) (Illumina, San Diego, CA, USA). The TES results revealed a heterozygous novel variant, c.95C>G (p.Pro32Arg), on *ELAC2* exon 1, which was confirmed by Sanger sequencing (Fig. 2A). This variant was predicted to be damaging using the *in silico* analysis (SIFT 0.029, prediction: damaging, MutationTaster 0.97, prediction: disease causing). The variant was detected in the control population with a frequency of 0.24% (KRGDB). The proline at position 32 is a highly conserved amino acid residue among different species (Fig. 2B). This mutation was inherited from her father. The additional variant could not be identified by Sanger sequencing.

Discussion

COXPD-17 is a very rare disease caused by mutation of the *ELAC2* gene on chromosome 17p12. The *ELAC2* was known only as a heritable prostate cancer-related gene[4,8], until it was first reported in 2013 to be associated with impaired OXPHOS in mitochondria[5]. Since the synthesized mitochondrial tRNA (mt-tRNA) is an unprocessed precursor, extra nucleotides must be removed to become a mature mt-tRNA[9]. The 3' end of tRNAs is mainly processed by the mitochondrial RNase Z, which is encoded by the *ELAC2* gene[10,11]. The study results by Haack et al.[5] indicated that impaired RNase Z activity due to the *ELAC2* mutation causes impaired OXPHOS resulting in cellular energy metabolism failure, especially by mitochondrial translation.

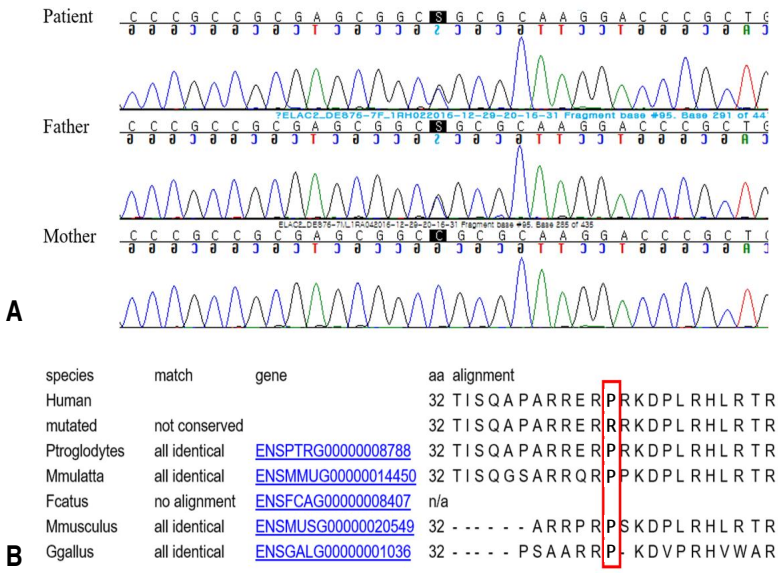

Fig. 2. (A) Direct sequencing of the proband and her father showing a novel heterozygous mutation of *ELAC2* gene, c.95C>G (p.Pro32Arg). (B) The p.P32 amino acid is completely conserved across different species, which is shown in red box.

Table 1. Summary of the patients with *ELAC2* gene mutation

Study	Number of patients	Zygosity	*ELAC2* gene	Clinical features	Course
Haack et al.[5] (2013)	German (2)	Compound heterozygous	c. 631C>T; 1559C>T (p.Arg211*; Thr520Ile)	HCM, IUGR, psychomotor and growth retardation, muscular hypotonia, microcephaly, dysphagia	Death at 6 months, alive at 2.10 years
	Arabic (1)	Homozygous	c.460T>C (p.Phe154Leu)	HCM, IUGR, muscular hypotonia	Death at 11 months
	Turkish (2)	Homozygous	c.1267C>T (p.Leu423Phe)	HCM, DCM, psychomotor retardation, muscular hypotonia	Alive at 13 years, death at 4.9 years
Akawi et al.[6] (2016)	Pakistani (5)	Homozygous	c.1423+2 T>A, splicing site mutation	Intellectual disability, mild septal hypertrophy	Alive at 2.5–19 years
Shinwari et al.[7] (2017)	Arabic (16)	Homozygous	c.460T>C (p.Phe154Leu)	HCM or DCM, developmental delay, seizures	Death at median age 4 months
Present case	Korean (1)	Heterozygous	c.95C>G (p.Pro32Arg)	Encephalopathy, cardiac anomaly, IUGR, growth retardation	Death at 5 months

HCM, hypertrophic cardiomyopathy; IUGR, intrauterine growth retardation; DCM, dilated cardiomyopathy.

Notably, COXPD-17 follows an autosomal recessive inheritance pattern, but in our patient, mutation in one allele was not found. Nevertheless, we strongly believe that the identified *ELAC2* gene mutation is closely correlated to the phenotype of the patient. The patient had hyperlactatemia which is indicative of mitochondrial dysfunction, and presented encephalopathy, suggesting significant involvement of brain in impaired mitochondrial function. There might be an additional mutation that has not been discovered by sequencing, because the possibility of a large deletion or duplication and an accompanying deep intronic mutation cannot be excluded.

Patients reported with *ELAC2* gene mutation showed mostly severe outcome with clinical manifestations that involve organs such as the brain, heart or muscle, which require high energy metabolism (Table 1). In this case, the main clinical issue of the patient was encephalopathy presented as central apnea, intractable epilepsy, and neurodevelopmental delay. She also had a cardiac anomaly without evidence of cardiomyopathy, and died of rapid deterioration of shock suspected of being caused by sepsis. According to the previous reports, most of the deceased patients with COXPD-17 died from cardiomyopathy, and no accompanying cardiac anomalies were reported[5-7]. Whether the cardiac anomaly in our patient was associated with COXPD-17 is unclear, it is rather thought to be less relevant given that mitochondrial dysfunction can lead to myocardial remodeling, which manifests as hypertrophic or dilated cardiomyopathy, and sepal hypertrophy[12,13]. In our patient, the reason for the absence of cardiomyopathy was presumably because she was in a relatively stable condition with medication and respiratory support.

In a study conducted in Saudi Arabia[7], homozygous p.Phe154Leu mutation in the *ELAC2* gene was found in 16 patients with severe infantile-onset cardiomyopathy, which was the same mutation as a previously reported patient[5]. The affected patients presented with hypertrophic or dilated cardiomyopathy between 2 and 7 months of age and all died with a median age of 4 months[5,7]. Moreover, 5 patients with a homozygous splicing *ELAC2* mutation (c.1423+2 T>A) in an Arabic inbred consanguineous family mainly presented intellectual disability, whereas cardiac involvement was not prominent, and all were alive at follow-up[6]. In this regard, it seems evident that the phenotype is correlated with a particular genotype of the *ELAC2* mutation.

In addition, a common characteristic among the affected patients was that lactate levels were high in all patients from whom serum lactate could be obtained[5,6]. Hyperlactatemia without any hypoxic condition indicates mitochondrial dysfunction, and it could be a surrogate marker of mitochondrial disorders. Mitochondrial disease should be considered as a likely cause in children with unexplained neurological deficit or involvement of organs such as heart or muscle, and it would be useful to identify hyperlactatemia as the first clue for mitochondrial dysfunction in these patients. Limitation of this study is that we could not measure the enzyme activity of mitochondrial respiratory chain complex I in muscle biopsy when mutation in one allele was not detected in the patient.

In summary, this is first case with COXPD-17 in Korea, which was diagnosed by clinical and molecular investigation, and revealed to involve a novel *ELAC2* gene mutation. Further study is required to understand functional and structural changes of proteins involved in this disorder and their associations with phenotypic spectrum.

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

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